NANOENCAPSULATION OF OPAA WITH MESOPOROUS MATERIALS FOR CHEMICAL AGENT DECONTAMINATION IN ORGANIC SOLVENTS

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ABSTRACT

Organophosphorus acid anhydrolase (OPAA) offers great potential for safe, non-corrosive decontamination of a wide range of organophosphorus compounds, including the fluoride containing chemical nerve agent Soman and Sarin. The nanoencapsulation of OPAA with mesoporous materials provides a very stable and convenient formulation for use in chemical agent detoxification. In the present study, the enzyme was prepared in both hydrophilic and hydrophobic matrices and measured for activity against diisopropyl fluorophosphate using a F^--specific electrode. The enzyme activity in different matrices was evaluated in various organic solvents. Significant activities were retained in most of these matrices, particularly in the presence of acetone and dimethyl formamide. These findings suggested that the OPAA-mesoporous hybrid materials might be suitable for a wide variety of applications including large area and personnel decontamination, individual protection, and detection.

INTRODUCTION

The use of biocatalytic enzymes as the new generation of chemical agents detoxificant has sparked a widespread interest. One enzyme, Organophosphorus Acid Anhydrolase (OPAA; EC.3.1.8.2), has been found in both bacteria and mammalian sources and has catalytic activity against many toxic organophosphorus compounds (OPs) and fluorine containing chemical nerve agents.[1-3] In 1989, DeFrank and Cheng isolated OPAA of *Alteromonas* strain (*A*. spJD6.5) from a warm spring of Utah. The gene encoding this enzyme has been cloned and sequenced. Biochemical analysis and gene sequence establishes this enzyme to be a prolidase, a type of dipeptidase that catalyzes the hydrolysis of P-F, P-O, P-CN, and P-S bonds commonly found in toxic OPs and G type chemical agents.[1,4]

Efforts to produce a large quantity of *A*. spJD6.5 OPAA with the goal of developing stabilized enzyme for long-term storage and decontamination have been successfully achieved[1,5]. The enzyme can be packaged as a dry powder form that could be reconstituted with various

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aqueous solutions. The OPAA enzyme is also stable in a wide variety of biodegradable yet water-soluble wetting agents or foams exhibiting no apparent loss of activity. These results clearly demonstrated the potential use of this enzyme with the existing fire-fighting equipment or spray apparatus for the safe and non-corrosive decontamination of chemical nerve agents. However, the poor stability and reusability of OPAA enzyme, particularly in harsh organic solvents, has become the major obstacle for field related applications.

In this study, a novel method to overcome the stability issue regarding the use of OPAA has been developed. It was shown that nanoencapsulated OPAA embeded in organically modified mesoporous materials can retain its activity in the presence of a number of organic solvents.

MATERIALS AND METHODS:

OPAA: Expression and purification of the gene encoding OPAA of *Alteromonas* JD6.5 has been reported.[1] All experiments in this study employed cloned, purified OPAA. The preparation of organic-inorganic hybrid mesoporous silica materials in the presence of either D(-)-fructose or polyethylene glycol (PEG) followed similar procedures as previously reported.[6,7] Five different nanoencapsulated-OPAA variations were prepared. All buffers and solvents were purchased from Sigma Chemical Company (St Louis, MO).

Enzyme assays: Activity measurements were performed using methods similar to those described elsewhere[1,4]. In general, a buffered solution of 50 mM 1,3 bis(trishydroxymethylmethylamino) propane (bis-tris propane) containing 1 mM $MnCl_2$, pH 8.5 was used in the assays for enzyme activity. When needed, 20% (V/V) of reagent grade organic solvent was added. The solution was placed in a beaker equipped with a magnetic stirrer and a fluoride sensitive electrode. The measurement proceeded in a thermally controlled water-jacketed reaction vessel maintained at 25 ^{o}C. Enzyme activity was measured by the rate of fluoride release in the presence of the substrate diisofluorophosphonate (DFP). One unit of enzyme catalyzes the release of 1 micromole of fluoride per minute at 25^{o}C. Specific activity is expressed as units per mg of enzyme after corrected for spontaneous hydrolysis under identical conditions.

RESULTS AND DISCUSSION:

Nanoencapsulated OPAA prepared in the various mesoporous matrix formulations were compared and analyzed in various solvent conditions. Table 1 summarizes the characteristics of the OPAA containing mesoporous materials. The pore size was controlled to be between 2-5 nm.

Table 1. Prepared Nanoencapsulated OPAA Sample Matrix Characteristics

Sample	**Precursor**	**Template**
A	TMOS/MTMS (1:3)	Fructose
B	TMOS/MTMS (1:3)	PEG
C	TMOS/MTMS (1:1)	PEG
D	TMOS	Fructose
E	TMOS	PEG

Legend: TMOS = Tetramethyl orthosilicate; MTMS = methyltrimethoxysilane, PEG = polyethylene glycol

Calculation of OPAA activity against DFP involved the equation:

Figure 1 compared the activity of free OPAA against various organic solvents relative to the enzyme activity in 50 mM Bis-Tris Propane at pH 8.5 (control). As expected, enzyme activity decreased in the presence of organic solvent.

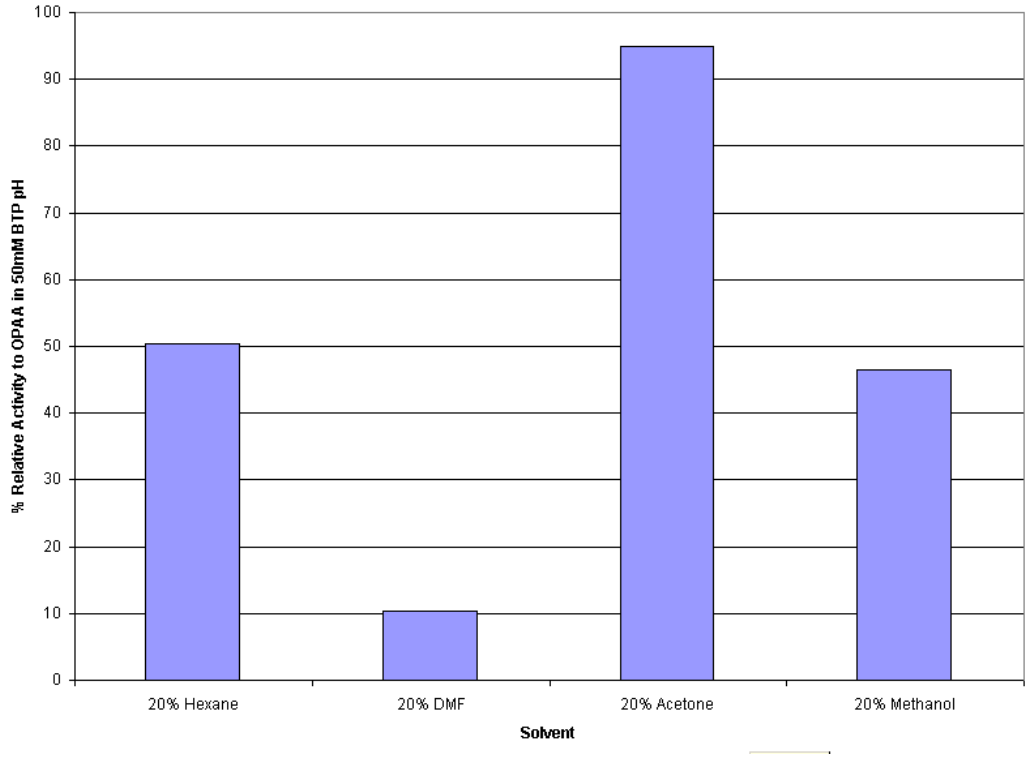

Figure 1. Enzyme activity of Free OPAA in Various Organic Solvents Relative to that in 50 mM Bis Tris Propane, pH 8.5 (control).

Figure 2 compared the nanoencapsulated OPAA activities in different organic solvents relative to native OPAA in control conditions as described above. The enzyme activity was greatly reduced in these solvents. Regardless of matrices, excellent enzyme activity was retained in the presence of these tested organic solvents. Except for hexane with significant enhanced activity, sample D also responded much better in the presence of DMF, acetone, and methanol when compared to its standard buffer conditions. Because of the limitation of the fluoride probe and the substrate (DFP) spontaneous hydrolysis rate in the organic solvents, the maximum solvent concentration that could be tested was 20%. At higher concentrations, the fluoride probe performance was greatly reduced. The observed excellent enzyme activity in organic solvents could be attributed to the fact that OPAA molecules are encapsulated within nanoscale-confined space of the mesoporous matrix. Because of the space confinement, the protein unfolding (i.e., denaturing) as caused by organic solvents has been significantly restricted and significant retention of enzyme activity is achieved[8-10]. Our preliminary results (data not shown) indicate that the nanoencapsulated enzyme is re-useable.

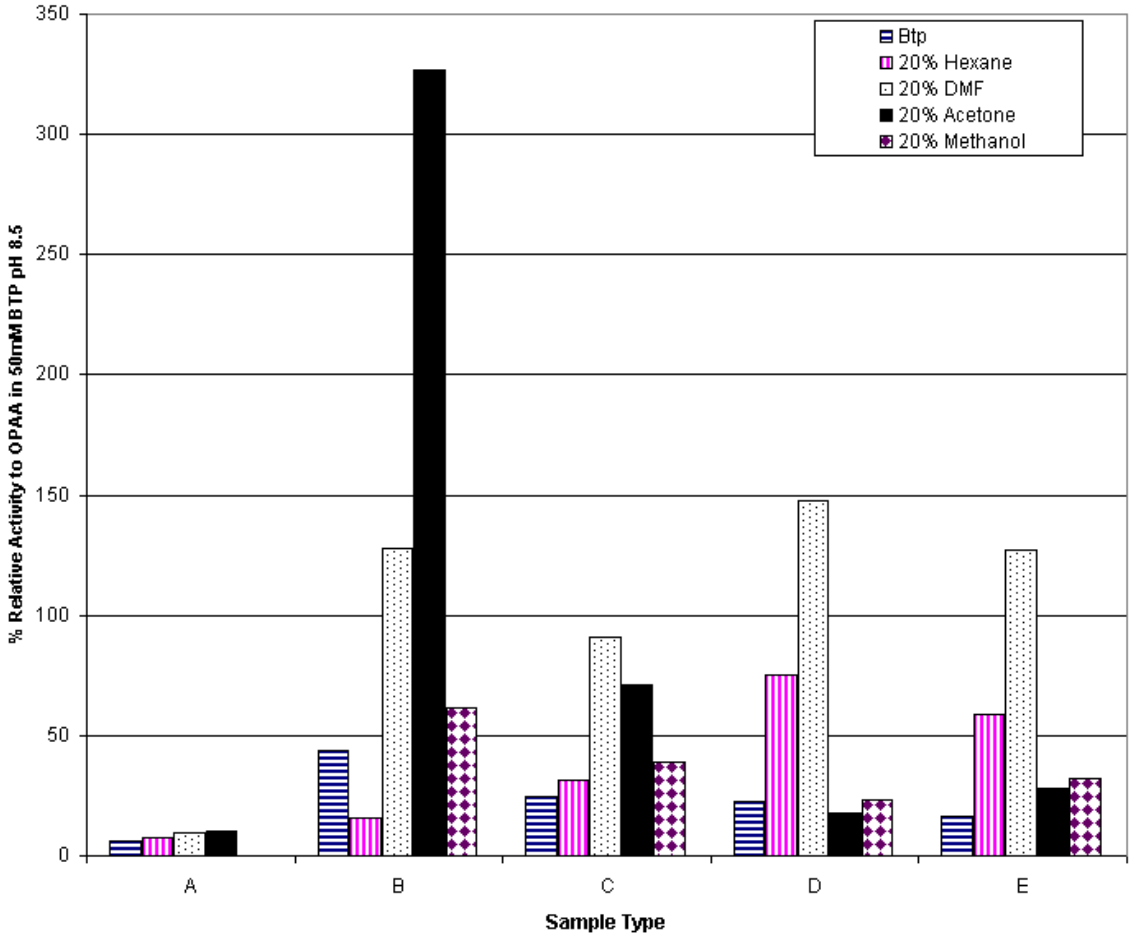

Figure 2. Comparison of OPAA Activities in the Five Different Sample Matrices and Solvents. Data presented are relative to native OPAA in 50 mM Bis Tris Propane, pH 8.5. Table 1 defines the different sample matrices (A, B, C, D, and E). The solvents studied are 50 mM Bis Tris Propane, pH 8.5 (horizontal-colored bars), 20% hexane (vertical-colored bars), 20% dimethylformamide (dot covered bars), 20% acetone (shaded bars), and 20% methanol (diamond covered bars).

CONCLUSIONS

Successful nanoencapsulation of OPAA in mesoporous materials was demonstrated. Enzyme activity was retained to a significant extent and in some cases, e.g., DMF and acetone, the activity was greatly enhanced compared to the free enzyme. Further studies are being conducted to test the stability and reusability of the nanoencapsulated enzyme as well as to elucidate the mechanism and pathway of OPAA folding and unfolding. The development of this novel materials system for decontamination and detection of toxic OPs and nerve agents is in progress.

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